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A Rare Mutation [IVS-I-130 (G-A)] in a Turkish β-Thalassemia Major Patient

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Here we describe the identification of the rare β-thalassemia mutation IVS-I-130 (G-A) for the first time in Turkey. The hematological evaluation of the patient showed classical signs of β-thalassemia major requiring regular blood transfusions every 30–35 days. DNA analysis was carried out using reverse dot-blot hybridization and restriction endonuclease digestion, as well as genomic sequencing. The patient was found to be heterozygous for the IVS-I-6 (T-C) and IVS-I-130 (G-A) mutations. In order to deduce a possible origin for the IVS-I-130 (G-A) mutation, the sequence polymorphisms in the DNA of the patient and her family were characterized. The method included the analysis of nine polymorphic nucleotides and the hypervariable microsatellite of composite sequence $(AT)_xT_y$ 5′ to the β-globin gene by DNA sequencing. The sequence haplotype (HT4) carrying the IVS-I-130 (G-A) mutation is also observed in Algeria. This favors a Northeastern African origin for this allele. The observed results agree well with a recent introduction of this mutation to Turkey from Egypt toward the end of the 19th century. Am. J. Hematol. 63:223–225, 2000. © 2000 Wiley-Liss, Inc.

Key words: β-thalassemia; rare mutation; haplotype; Turkey; North Africa

INTRODUCTION

More than 180 different mutations resulting in a β^0- or β^+-thalassemia phenotype have been identified in many populations of the world [1]. Molecular studies of β-thalassemia in Turkey recorded the presence of 37 different alleles associated with the disease [2–5]. These studies also revealed that several of these alleles are either rare or not described in other populations. Here we describe the identification of the rare mutation IVS-I-130 (G-A) for the first time in a β-thalassemia major child from Turkey.

MATERIALS AND METHODS

Patient

The patient is a 4-year old Turkish girl from Istanbul (A.K.); she presented two years ago at the Pediatric Hematology Division of Istanbul University Medical School because of paleness, poor appetite, and no gain of weight. The hematologic evaluation of the patient showed classical signs of β-thalassemia major (Table I). HbA_2 was detected by commercial column chromatography and HbF by HPLC. After the definite diagnosis, she received two blood transfusions, 3 months apart. Since then, her clinical and laboratory data have necessitated regular blood transfusions every 30–35 days.

DNA Isolation and Analysis of Mutations

Blood samples from the patient and members of her family were collected in EDTA-containing tubes. Informed consent was obtained. DNA was extracted from white blood cells according to the method of Poncz et al. [7]. Screening for the most common Mediterranean mu-

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TABLE I. Hematological Data of the Proposita and Her Family Compared to Those of the Egyptian Patient Described by Deidda et al. [6]

Subject relationship genotype	C.K. Father IVS-I-6/β^A	I.K. Mother IVS-I-130/β^A	D.K. Twin brother IVS-I-6/β^A	A.K. Proposita IVS-I-6/IVS-I-130	N.G. Grandmother IVS-I-130/β^A	Egyptian patient IVS-I-6/IVS-I-130
Hb (g/dl)	14	9.2	–	7.4	–	8.6
Hct (%)	41.2	26.4	–	21.1	–	25
RBC (10^{12}/L)	6.06	4.78	–	3.52	–	3.76
MCV (fL)	68	58	–	60	–	–
MCH (pg)	23.1	19.2	–	21	–	–
MCHC (g/dL)	34.0	33.2	–	35.1	–	–
Ret. (%)	5	8.6	–	7.6	–	–
HbA_2 (%)	4.4	6.6	7.4	1.8	6	4.2
HbF (%)	1.05	2.6	1	59	1	40

tations was carried out using a commercially available mutation detection system (β-Globin StripA^{ssay} Kit; ViennaLab, Austria), based on reverse dot-blot principles [8]. The test strip contained an array of nine oligonucleotide probe pairs covalently immobilized on a membrane support as parallel lines; these were as follows: −87 (C-G), HbC, HbS, IVS-I-1 (G-A), IVS-I-6 (T-C), IVS-I-110 (G-A), Cd39 (C-T), IVS-II-1 (G-A), and IVS-II-745 (C-G). Further analysis included the PCR amplification of the region extending between the positions +26 and Cd 49 of the β-globin gene using the primers PCO3 (5′-ACA CAA CTG TGT TCA CTA GC-3′) and Cd39N (5′-CAG ATC CCC AAA GGA CTC AAA GAA CCT GTG-3′). DdeI digestion was carried out on the amplified products to exclude the possibility of the common FSC-5 (-CT) and FSC-6 (-A) mutations, the probes of which are not present on the membrane. This analysis showed an unexpected pattern due to an abolished DdeI site that was calculated to be at the acceptor splice site of IVS-I. Subsequently, amplified β-globin genes of the patient and members of her family were directly sequenced by the method of Sanger et al. [9] using α-[^{35}S]-dATP (Izotop, Hungary) and Sequenase Version 2.0 (USB Corp.).

Haplotype Analysis

The patient's and her family's DNA samples were subjected to haplotype analysis as described by Trabuchet et al. [10,11]. The method included the sequencing of a 790-bp DNA fragment, located 400 bp upstream to the β-globin gene [12]; nine polymorphic nucleotides and a hypervariable microsatellite of composite sequences $(AT)_xT_y$ were analyzed in this region.

RESULTS

Analysis of Mutations

Using the β-globin StripA^{ssay} Kit, one allele of the patient was found to be IVS-I-6 (T-C). The mutation was confirmed to be also present in the DNA of her father (C.K.) as well as in her twin brother (D.K.). DNA sequencing for the other allele uncovered the presence of the rare IVS-I-130 (G-A) mutation in the patient, her mother (I.K.), and her grandmother (N.G.; Table I).

Haplotype Analysis

Sequence analysis of the polymorphic nucleotides −1069, −989, −780, −710, −703, −551, −543, −521, −491, and the hypervariable microsatellite of composite sequences $(AT)_xT_y$ 5′ to the β-globin genes of the proposita and her family showed the occurrence of a distinct haplotype for each of the two mutations IVS-I-6 (T-C) and IVS-I-130 (G-A) (Table II).

In an effort to trace back the origin of the IVS-I-130 (G-A) mutation in the family of our patient, we studied samples belonging to members of her mother's family and found that the mutation was inherited from her grandmother. An investigation of the origin of the grandmother's parents, showed that they were Albanians who immigrated to Turkey, most probably, early in this century.

DISCUSSION

At present, more than 180 mutations, causing β-thalassemia, have been identified at different positions within the β-globin gene itself or its immediate flanking sequences [1]. Only three of these mutations occur at the acceptor site of the IVS-I of the β-globin gene; namely, IVS-I-128 (T-G) [13], IVS-I-130 (G-C) [16,17], and IVS-I-130 (G-A) [6]. To our knowledge, the latter mutation was described only once in an Egyptian patient from the city of Mansoura [6]. This patient carries the IVS-I-130 (G-A) lesion along with the Mediterranean IVS-I-6 (T-C) β-thalassemia mutation. The patient we describe in the present report has also heterozygously inherited the same two mutations. Such a coincidence allowed us to compare the phenotypes of the two patients and conclude that their hematological data were almost similar with the exception that the Egyptian patient [6] had an increased level of HbA_2 (4.2%) whereas our patient's HbA_2 is 1.8%. Both patients, however, had remarkably increased levels of HbF (40 and 59%).

TABLE II. Sequence Polymorphisms Observed in the IVS-I-6 (T-C) and IVS-I-130 (G-A) Mutations Compared to the Reference Sequence Haplotype (HTR) [13] and That of the Algerian IVS-I-2 (T-C) Mutation [14]†

Genotype	HT	−1069	−989	−780	−710	−703	−551	−543	$(AT)_xT_y$	−521	−491
−28 (A-C)	HTR	G	C	A	T	T	T	C	$(AT)_7T_7$	C	A
IVS-I-6 (T-C)	HTR	*	*	*	*	*	*	*	*	*	*
IVS-I-130 (G-A)	HT4	*	*	*	*	C	C	*	*	*	*
IVS-I-2 (T-C)	HT4	*	*	*	*	C	C	*	*	*	*

†Polymorphic sites are numbered in relation to the β-globin cap site according to Trabuchet et al. [10]. The * symbol indicates homology with the corresponding reference sequence haplotype position.

In recent years, DNA sequence variation in the intergenic domain upstream of the β-globin gene has attracted an increased attention. A large group of studies have accumulated in the last few years, most of which aimed at deducing the possible origins of some β-globin gene mutations through the analysis of several nucleotide polymorphisms and the $(AT)_xT_y$ motif 5′ to the β-globin gene [10–12,18,19]. In order to deduce a possible origin for the IVS-I-130 (G-A) mutation, we have characterized sequence polymorphisms in the DNA of our patient and her family. The IVS-I-6 (T-C) mutation was found to be associated with the expected reference sequence haplotype (HTR) of Poncz et al. [13], frequently observed in wild-type β-globin chromosomes from the Mediterranean [12,20]. The IVS-I-130 (G-A) mutation, on the other hand, was present on a less common sequence haplotype (HT4) [20]. The haplotype HT4 seems to be mainly associated with the Algerian IVS-I-2 (T-C) mutation [12,14]. In conjunction with this, the fact that the mutation IVS-I-130 was described once in an Egyptian patient [6] could be a good indication favoring a Northeastern African origin. In fact, it is known that many Turkish families originating from Albania settled in Egypt until the end of the Ottoman rule (1805–1882 A.D.) [21]. The mutation may have well migrated into Albania during this time.

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